Title: Prevalence of dyslipidaemia in HIV-infected children and adolescents treated with protease inhibitors

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RESPONSE TO EXAMINER'S COMMENTS:

The second protocol study objective of comparing the incidence/prevalence of dyslipidaemia in different age groups was not fully met and the reason that this wasn't done is not elaborated on in the paper. The age groups were changed to < 10 years and > 10 years. I feel that it would be beneficial to keep the original age groups and compare children 0-5 years, 5-10 years and 15-19 years as these age groups are likely to have been exposed to different ART regimens and to have different disease profiles. If the numbers were too small, then the age breakdown of the children should be more clearly explained as the range is too broad.

I couldn't meet this objective because of the large number of missing variables making it impossible to do the analysis for 4 groups. For example, at baseline (ART initiation) only 7% (146/2145) patients had any lipids measured, with no lipid measurements in the 17 patients 15-19 years old (see results paragraph 2). For that reason, I limited the analysis to 2 age groups: children (<10 years) and adolescents (≥ 10 years). The age range is explained in methods, paragraph 6 and addressed in the study limitations (see discussion, paragraph 6). All edits in blue for easy reference.

ABSTRACT

The abstract is concise. It is difficult to understand if the paper hasn't been read first as it is not explained what is meant by 12 and 24 months. This needs to be clarified to improve understanding: "12 and 24 months after starting LPV/r."

Agreed. I have made the suggested edits on the attached abstract.

It is stated that the median age at ART-start was 1.6 years....

Agreed. I have added this important information in the results section of the paper, paragraph 1.

It states: "At 24 months on LPV/r, ART duration greater than 60 months was an *additional* protective factor." Either additional should be removed or high VL added.

I have added high VL.

It would be relevant to describe how many children out of the potential population actually were included in the study and how many children had no data.

I have included this data in the results section.

INTRODUCTION

I feel that it would be relevant to discuss the effects of NRTI's such as D4T, AZT and DDI on lipid profiles as many children were likely on these NRTIs.

The patients were on varying NRTI backbones. In the initial analyses I stratified based on the NRTI backbones, those that are associated with dyslipidaemia (stavudine, zidovudine, didanosine) and those that are not associated with dyslipidaemia (lamivudine, abacavir and tenofovir); I found no association. Low numbers could have been a limiting factor. In the end I decided to focus on LPV/r and LPV/r with additional ritonavir as boosting for rifampicin-based TB treatment. The time periods (before or after 2010) are essentially a proxy for the different NRTI backbones as described above. However, since I found no statistical difference using the NRTI backbones themselves, a time-based analyses is not warranted. The suggested corrections were made in paragraph 4 of the introduction, paragraph 1 of methods, paragraph 10 of results and paragraph 5 of the discussion.

The normal effect of age on lipid profiles should also be discussed as these children were a wide age range (Birth-19 years).

This study focused on the effect on lopinavir/retinovir (LPV/r) on dyslipidaemia. In the introduction I mention that there are physiological changes in lipid profiles in children. I have expanded on what these changes are (paragraph 4).

METHODS

The age range of the study is not clearly stated except in the first table.

Age range added in the methods section, paragraph 3.

How many children were potentially on d4T, AZT or DDI for the entire 24 months period? These NRTIs have all potentially been linked with dyslipidaemia and it would therefore be important to document the NRTI backbone if possible or to compare children on treatment before 2010 to those on treatment after 2010.

I am not able to report on the proportion of children on d4T, AZT or DDI for the study period as I no longer have access to the data. However, in the analysis based on NRTI backbone I found no association with dyslipidaemia at all 4 time points.

RESULTS

It needs to be clearly explained both in Table 1 and in the first paragraph, when the data was captured.

Suggested edits made in Table 1 and results section of the paper, paragraph 1.

'Demographic and clinical characteristics of 2145 children started on a lopinavir/ritonavir containing regimen at ART start (either NNRTI or PI based)'. I am not sure if the table reflects data at LPV/r start. How many children was 1st ART start=LPV/r start? There is no explanation for # in the table

Suggested edits and clarification made in Table 1 and results section of the paper, paragraph 1.

It is reassuring that children who did and didn't have lipids measured were similar. It would be important to make sure that they were also on similar NRTI backbones.

I am not able to report on this as I no longer have access to the data.

It is confusing that paragraph 1 states that anthropometric z-scores were normal at ART start whereas paragraph 3 says that in children who had lipids taken, 70% of the children were underweight and stunted at ART and LPV/r start! This needs to be explained as the sensitivity analysis suggests that there is no difference in anthropometry between those who had lipids measured and those who didn't.

Overall the anthropometric z-scores were normal at ART start. However, the age group <5 was the only group that was stunted and underweight (see Table 1). I postulate that this was due to the young children even though the sensitivity analysis doesn't show any significant age difference between the children with measured lipids and those without at these 2 time points. This is highlighted at the end of paragraph 1, results section.

The degrees of underweight, stunting and BMI are also slightly different in the ART and LPV/r start groups not the same as suggested in the text.

The table refers to normal anthropometry, with the abnormal (underweight, stunted and wasted) inferred. The paper speaks to the abnormal anthropometry. I have edited the text to correlate with the table.

Table 2 is missing the m after 24 in the heading.

Corrected.

Dyslipidaemia is clearly defined but doesn't explain figure 1 and supplementary figure 2. If dyslipidaemia is defined as hypercholesterolaemia and/or hypertriglyceridaemia how is it possible for the percentage of patients with dyslipidaemia to be less than the number with hypertriglyceridaemia?

I have verified all the numbers, and it's correct. The reason the hypertriglyceridaemia is higher than the dyslipidaemia is because of different denominators. For example (refer Table 2) at ART start, there are 133 measured triglycerides vs 146 measured cholesterols; thus the dyslipidaemia is calculated with a higher denominator. Similarly, for the small cohort of children with measured lipids at all 3 time points: there were 16 measured triglycerides vs 19 measured cholesterols.

If there is uncertainty about the definition of dyslipidaemia, what definition was used to do the multivariate analysis? Was separate analysis done for triglycerides and cholesterol or

were they combined into one category ‘dyslipidaemia’ consisting of either AND/OR one of them? This needs to be clearly defined.

The definition of dyslipidaemia given in the methods section, paragraph 5 was used in all multivariate analyses.

It would be interesting if the group with lipids measured at all four time points could be better described mentioning such things as age, viral suppression and NRTI backbone.

The cohort of children with measures lipids at all 3 time points: the age is mentioned in paragraph 5. I am unable to report on the viral suppression and NRTI background as I no longer have access to the data.

Table 3 is a little confusing. If ART start was = LPV/r start were children included in both groups? How many children had ART start = LPV/r start?

ART start is only the same as LPV/r start in the cohort of children who had all measured lipids. However, outside of this cohort for those children who had ART start = LPV/r start they were included in both groups. The number of children who had ART start = LPV/r start is shown in Table 2.

Why is BMIZ score for children > 10 years at ART start empty?

There were very few measured lipids in this group of children to allow for proper analysis (only 6 measured lipids in the normal BMIZ category)

DISCUSSION

The fact that a suppressed viral load was associated with increased risk of dyslipidaemia seems to be contradictory to the fact that ART duration greater than 60 months was a protective factor. This could be better discussed – was it due to different ART exposure?

A possible explanation for this finding is offered in paragraph 4 of the discussion.

The long term effects of increased total cholesterol with an improved triglyceride profile are unknown and the fact that only total cholesterol was measured and not LDL or other factors such as inflammatory markers that are better linked to cardiovascular outcomes was not discussed. The improvement of triglycerides on cardiovascular outcomes also wasn’t discussed.

Agreed. This is briefly mentioned in paragraph 4 of the discussion. I have expanded on this in paragraph 5.